Growing wheat

This document explains how to germinate and grow wheat, as well as how to harvest and store the grain. As mentioned in the **Introduction**, wheat varieties can be categorized into two groups according to their requirement for sensing cold before flowering (vernalization requirement). This is also used to classify wheat lines as winter sown (requires vernalization) or spring sown (does not require vernalization). The TILLING populations have been developed in the cultivars Cadenza and Kronos, both of which do not require vernalization.

a) Germinating grain

Grain can be germinated directly in soil but germination is less uniform with this approach; place the grain 3-5 cm under the surface of the soil and ensure it is well watered.

Alternatively, a more uniform germination can be achieved by germinating grains on filter paper before transferring them into soil.

Materials needed

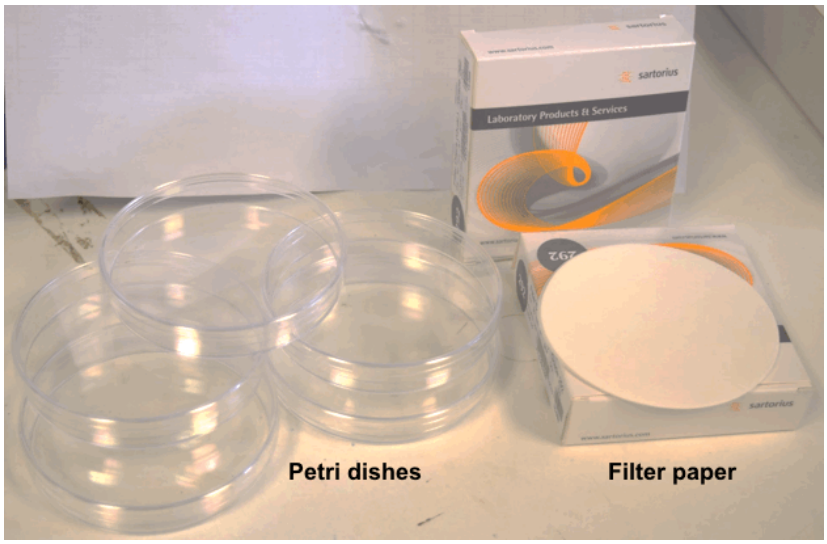

Figure 1. Petri dishes and filter paper for germinating grain

- Mature, dry, properly stored grain (see **harvesting and storage** section)
- Petri dishes (we use either 5-cm or 9-cm diameter dishes)
- Filter paper (to fit in Petri dishes)
- Water (sterile distilled water, or normal tap water)
- Fridge or 4°C cabinet
- Permanent marker

Method

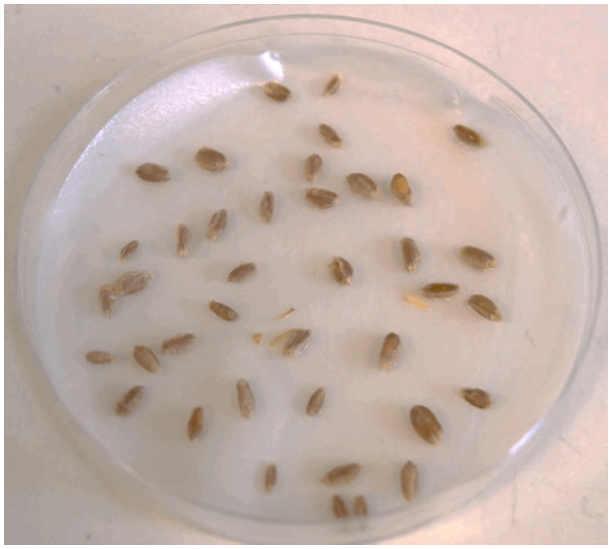

Figure 2. Grain imbibing on wet filter paper in large Petri dish

- If the grain has been stored at room temperature, then placing them at 4°C for 24 h first aids germination. To germinate prematurely harvested grain, see the **Speed Breeding section**.
- Place a circle of filter paper in a Petri dish and put grains on top, in small Petri dishes use about 15, in large Petri dishes about 40.
- Label dishes carefully, preferably not on the lids. We usually also label the filter paper with pencil.
- Add enough water to dampen the filter paper completely, but make sure the grains are not floating in water.
- You can seal the lid closed (with sticky or masking tape) to prevent the emerging coleoptiles from pushing the lid off, which can lead to the filter paper (and thus the grains) drying out.
- Leave at room temperature for 3-5 days until germinated (i.e. the coleoptile has clearly emerged), but check periodically that the filter paper is not drying out. Some varieties germinate better if left at 4°C rather than room temperature. Germination can be sped up by placing in a germinator (under constant light).
- In general, the older the grains are the longer they should be left at 4°C before moving them back to room temperature. This drastically increases the germination rate of old(er) grains.

b) Transferring germinated grain to soil

Once the grains have germinated they can be transferred to soil. Normally we transfer them to 96-cell trays first. This is convenient because we often perform genomic DNA extractions in 96-well plates so plants and tissue samples are arranged in the same pattern.

Materials needed

- Multi-cell seed tray (96 cell pots is a good size, with 5cm^3 volume for a single cell, **Figure 3A**)
- Pot labels
- Soil levelling tool
- Permanent marker
- Suitable soil. We use the following Peat Based F2, but anything similar will do:
    - 85% fine peat
    - 15% grit
    - 2.7 kg/m^3 maglime
    - 1 kg/m^3 PG mix (premixed fertiliser)

Method

- Fill a multi-cell seed tray with Peat Based F2 mix. Use a levelling tool (or your hands) to pat down the soil and then add more until the tray is almost full.

Alternatively, you could use John Innes Cereal mix soil (see next section); since this soil is much heavier than the Peat Based F2 mix you need to be extra careful not bury your emerging coleoptile.

- Use a finger to scrape a small well in the soil, with a mound next to it.
- Place the germinated grain in the well and push the small mound of soil over it, so that the grain is covered but the coleoptile is not buried.
- Use pot labels to make sure you know what each plant is.
- Water the soil thoroughly without flooding.
- Place the tray in an appropriate growth chamber or glass house for two weeks.
- Once the first true leaves have formed it is a good time to take samples for genomic DNA extractions. We usually put leaf samples in a 96-well plate in the same order as the plants in the seed tray. At this stage the plants are easy to sample and the leaf material is soft and easy to disrupt.

c) Vernalization requirements (Winter wheat varieties)

Winter wheat varieties will require between 6 to 12 weeks of vernalization, the exact time depending on the specific variety. Plants are usually vernalized in a cold room at 4 °C and are placed there straight after having germinated or once the first true leaf has formed. This allows for DNA sampling before putting them into vernalization. The required temperature and time period for vernalization is closely related to the winter hardiness and maturity rating of the variety.

d) Transplanting from trays to pots

Once the plants are big enough we transfer them to pots. They remain in these pots until they set seed.

Depending on your experiment and your available space, you could also leave your plants in 96-cell trays; they should produce at least one or two healthy spikes. In this case you should have used a nutrient rich soil mix, e.g. John Innes Cereal mix.

Materials needed

- One-litre pots
- Pot labels
- Suitable soil. We use John Innes Cereal mix, but anything similar will do:
    - 40% medium grade peat
    - 40% sterilised soil
    - 20% horticultural grit
    - 1.3 kg/m^3 PG mix 14-16-18 + Te base fertiliser
    - 1 kg/m^3 osmocote mini 16-8-11 2 mg + Te 0.02% B
    - Wetting agent
    - 3 kg/m^3 maglime
    - 300g/m^3 exemptor

Method

- Fill a 1-L pot to approximately ¾ full.
- Remove the entire plant from the multi-cell seed tray (try to not disturb the roots) and place in the pot (**Figure 3B** and **C**).
- Put more soil over the top, ensuring that the roots are covered and the shoots are not buried, and gently press with your fingers (**Figure 3D**).
- Make sure that all pots are clearly labelled (**Figure 3E**).

- Place plants in an appropriate growth chamber or glass house; we place approximately 40 pots per square metre.

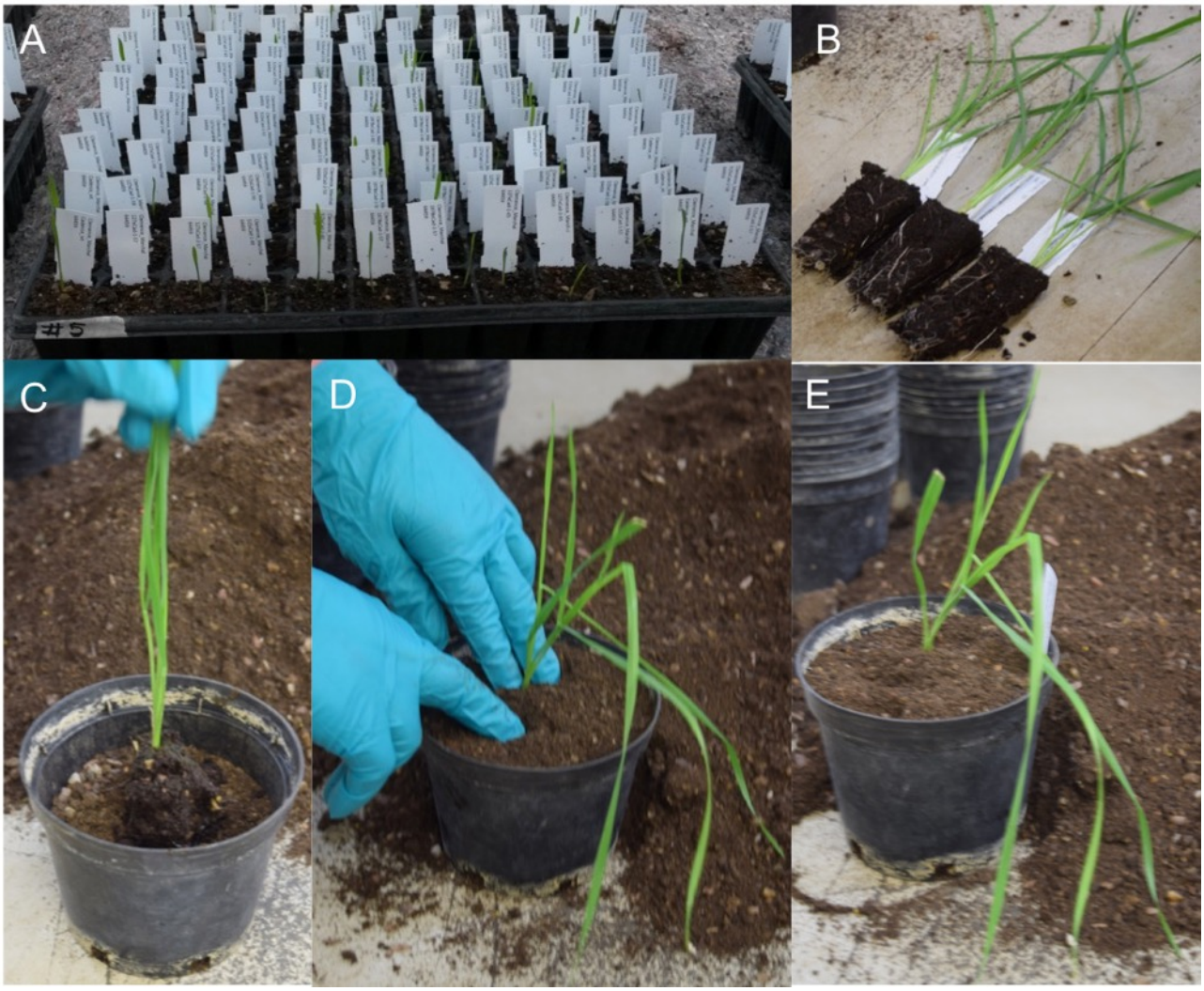

Figure 3. Potting up wheat plants

e) Growth conditions

- Plants can be grown in controlled environment rooms on a 16 h light (300 µmol m^{-2} s^{-1}) 20°C / 8 h dark 15°C cycle with constant 70% humidity. We also grow plants under accelerated growth conditions which is described in the **Speed Breeding section**.
- We generally grow the plants in a glasshouse. During the winter these are augmented with lights and heaters, to give conditions similar to those described for the controlled environment rooms.
- Plants are watered 2-3 times per week; make sure that the pots do not dry out. Watering is reduced when the grains mature and stopped altogether once the plant has senesced.
- Once bolting occurs plants are staked and tied to prevent them from falling over or lodging (**Figure 4**).

- Invigorator can be applied (to kill pests including mildew) – follow manufacturer's instructions.

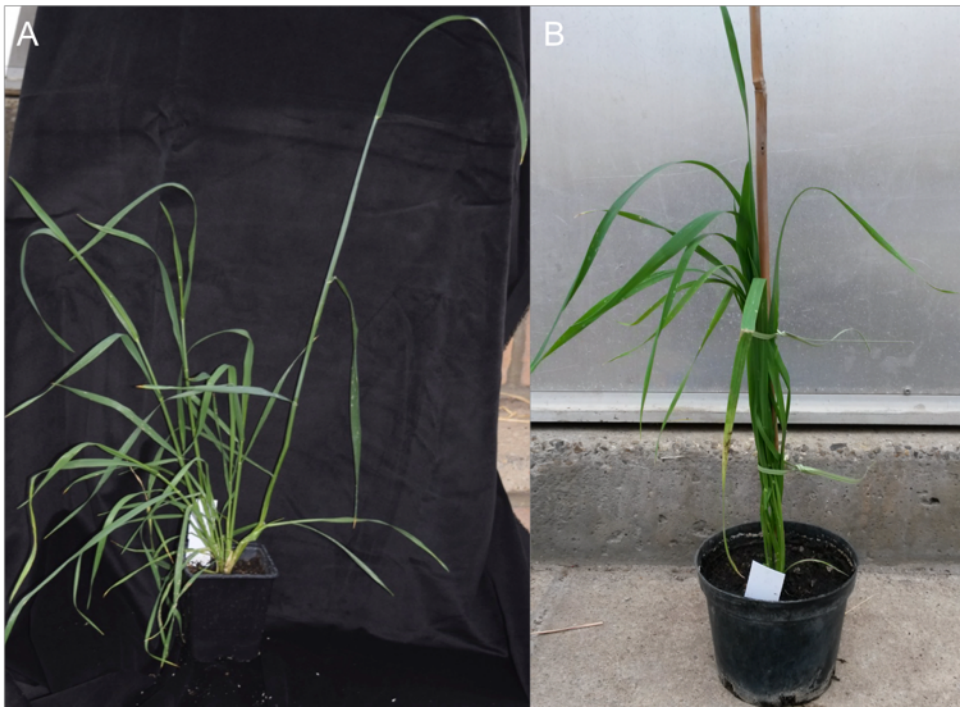

Figure 4. Bolting plants can be staked and tied to prevent falling over.
(A) Plant falling over, (B) staked and tied plant

f) Harvesting and storing of grain

After anthesis the grain begins to form quite rapidly for about ten days (the "pre-milk" stage). After its formation the grain is gradually filled (grain filling stage), which takes approximately 20 days (each variety will be somewhat different). Towards the end of this period the grain turns from green to a golden colour. After this period the grain begins to dry out and its colour becomes brownish.

Grain can be harvested when completely dry or collected earlier and dried in the oven (30-35°C; do not cook your grain!). In the case of speed breeding, grains are collected very prematurely (see **Speed Breeding section**). Before storage, the grains must be "threshed", i.e. separated from the rest of the spike. This can be done in a number of ways but we use a cricket bat grip (made of rubber). **Figures 5** and **6** illustrate the required tools and the procedure.

After threshing we store the grain in envelopes (clearly labelled, including date, and fastened with staples or a paperclip). Envelopes are better than tubes because tubes can trap moisture which can cause the grain to go mouldy. In the short term, grain can be stored at room temperature but for long term storage place the seeds at 4°C.

Figure 5. Threshing toolkit

Figure 6. Threshing wheat
(A) Place the wheat spike inside the cricket bat grip and (B) roll the cricket bat grip with your hand. Make sure to fold the ends over to prevent seeds and chaff from spilling out! (C) Pour seed and chaff onto the plate. (D) At this step, you can use a hood with a vacuum system to remove the chaff, which is lighter than the seed, or just separate the seed from the debris by hand. (E) Finally pour the seed in a labeled envelop (F).

Note: See Speed-Breeding document for growing conditions shown to shorten wheat generation time.

g) Glossary of terms

Anther	**The male (pollen-producing) part of the flower**.

Anthesis	Time that the anthers become mature and **pollination** takes place. Anthesis begins first in the **florets** at the centre of the spike and then moves outwards towards the base and the tip of the spike.
Awn	The bristles on the wheat ear, extending from the **florets**. Awn length varies with variety; most UK bread wheat varieties have no awns.
Coleoptile	Protective sheath covering and protecting the young shoot tip.
Emasculation	Removal of the **anthers** (male parts) from the flower to prevent self-**pollination**.
Flag leaf	The last leaf to emerge before the spike; it sits directly below the spike. Like every leaf it is composed of the leaf sheath (the part wrapped around the stem) and the leaf blade.
Floret	Each individual flower (containing anthers and stigma). Several **florets** (usually three to five) form a single **spikelet**.
Heading	The growth stage when the ear pushes out from the **flag leaf sheath**. We define a plot as "**heading**" when 75% of the spike is visible in 75% of the plants have.
Imbibition	The act of the seed absorbing water prior to germination. Seeds become fully metabolically active again after imbibition.
Ovary	The immature un-pollinated grain precursor. It consists of the embryo surrounded by endosperm.
Peduncle	The stalk carrying the spike. Only a small part of the **peduncle** is exposed, while most of it is hidden underneath layers of leaf sheaths.
Pollination	Pollen deposition onto the stigma; this leads to fertilisation of the ovum and thus to a new seed.
Radicle	The first part of the growing seedling to emerge from the seed coat, later becoming root tissue.
Senescence	Aging of the plant; the process begins when the new grain starts to form; the colour of the plant changes from green to gold/yellow.
Spike	The wheat ear. A spike usually consists of ~20 **spikelets**.
Spikelet	The basic unit of a wheat flower. Each spikelet consists of at least three **florets**.
Stigma	The female (pollen-receptor) part of the flower sitting on top of the ovary.
Tiller	A shoot originating from the shoot meristem at the base of the plant.
Vernalization	Exposure of the plant to cold temperatures to induce flowering. This mimics the winter season and serves as an environmental cue for plants to flower; essential for winter wheat varieties.

References

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2. Ghosh S., A. Watson, O.E. Gonzalez-Navarro, R.H. Ramirez-Gonzalez, L. Yanes, M. Mendoza-Suárez, J. Simmonds, R. Wells, T. Rayner, P. Green, A. Hafeez, S. Hayta, R.E. Melton, A. Steed, A. Sarkar, J. Carter, L. Perkins, J. Lord, M. Tester, A. Osbourn, M.J. Moscou, P. Nicholson, W. Harwood, C. Martin, C. Domoney, C. Uauy, B. Hazard, B.B.H. Wulff, L.T. Hickey (2018). "Speed breeding in growth chambers and glasshouses for crop breeding and model plant research". Nature Protocols. **13,** 2944–2963, DOI